



UNIVERSITY OF GONDAR  
COLLEGE OF VETERINARY MEDICINE AND ANIMAL SCIENCES  
DEPARTMENT OF PARACLINICAL STUDIES

EPIDEMIOLOGY AND SPECIES IDENTIFICATION OF IXODIDATICKS ON CATTLE IN  
THREE SELECTED DISTRICTS OF NORTH GONDAR ZONE, NORTHWEST ETHIOPIA

BY  
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A thesis submitted to the College of Veterinary Medicine and Animal Sciences, the University of Gondar for partial fulfillment of the requirements for the Degree of Master of Science in Veterinary Parasitology

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**UNIVERSITY OF GONDAR**  
**COLLEGE OF VETERINARY MEDICINE AND ANIMAL SCIENCES**  
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## **LIST OF ABBERVATIONS**

BCS	Body condition score
CSA	Central Statistical Authority
SPSS	Statistical package for social science

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## ABSTRACT

A cross-sectional study was conducted from December 2016 to April 2017 in three purposefully selected districts (Wegera, Gondar Zuria and Chillga) of North Gondar Zone, Amhara Region, Northwest Ethiopia. The objectives of the study were to estimate the prevalence of hard tick infestation and to identify the tick species prevalent in the study areas. A total of 384 cattle (comprising 283 local and 101 cross breeds) were randomly selected (128 cattle for each district) and examined for the presence of ticks infestation. Out of the total examined animals, 215(56%) were found to be infested by one or more tick species. Four genera of ticks: *Amblyomma*, *Boophilus* (recently *Rhipicephalus*), *Rhipicephalus* and *Hyalomma* were identified. *Amblyomma* was the most dominant tick genera 68.16 % (n=1473) while *Hyalomma* 2.96% (64) was the least recorded genera in the study areas. Within the tick species identified, *Amblyomma varigatum* was the most abundant tick species found in the present study 57.3% (n=556) followed by *R(B). decoloratus* 17.95%, *A. cohaerence* 17.81%, *R. evertsi evertsi* 10.90%, *A. lepidum* 3.70% and *H. marginatum* 2.96%. However, in highland (Wegera) *A. cohaerence*, *A. lepidum* and *H. marginatum* were not recorded. The overall prevalence in relation with agroecology was significantly higher in lowlands and midlands than highland areas ( $P < 0.05$ ). The sex-wise prevalence of tick infestation was not statistically significant ( $P > 0.05$ ) (female 57.20% and male 54.05%). Breed, age, localities, body condition score and months were significantly ( $P < 0.05$ ) associated factors with tick infestation in cattle. Tick infestation was higher during April while, it was lowest during February. Therefore, the higher occurrence of different species of ticks in lowland and midland agroecologies as well in cross breed cattle necessitates control measures against economically more important tick species.

**Keywords:** Ticks, Cattle, , Prevalence, , North Gondar

## 1. INTRODUCTION

Ticks are ectoparasites of livestock, which are classified (together with mites) in the order Acari. All ticks are **obligate ectoparasites** of vertebrates. They have three pairs legs in larvae and four pairs of legs as nymphs and adults and the body is divided into the capitulum and the opisthosoma. There are at least 840 tick species in two major families, namely the Ixodidae and Argasidae, (Jongejan, F. and G. Uilenberg, G.1994)

Ticks are very significant and harmful blood sucking external parasites of mammals, birds and reptiles throughout the world (Mesele *et al.*, 2010). Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing a considerable portion to the economy of the country and still promising to rally round the economic development of the country. In Ethiopia livestock production remains crucial and represents a major asset among resource-poor smallholder farmers by providing milk, meat, skin, manure and traction force (Abdela *et al.*, 2016). Ticks have considerable impact on animals either by inflicting direct damage or by transmission of tick-borne pathogens. Tick and tick born disease affect 90% of the world's cattle population and are widely distributed throughout the world (Estrada *et al.*, 2014). The country's environmental condition and vegetation are highly conducive for ticks and tick-borne disease perpetuation (Abdela and Bekele, 2016).

The presence of diseases caused by haemoparasites is broadly related to the presence and distribution of their vectors. Ticks are more prevalent in the warmer climates, especially in tropical and sub-tropical areas (Ikpeze *et al.*, 2015). Ticks are considered to be most important to the health of domestic animal in Ethiopia. Ticks comprise various type of genera, including *Amblyomma*, *Rhipicephalus*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus (Boophilus)*. The genus *Amblyomma* and *Rhipicephalus (Boophilus)* are predominating in many parts of country (Eyob *et al.*, 2015).

Tick borne haemoparasite diseases of ruminants such as *Anaplasmosis*, *Babesiosis* and *Theileriosis* remain more important in tropical countries. The effects of ticks estimated annual loss of US\$ 500,000 from hide and skin downgrading and approximately 65.5% of major defects of hides in Eastern Ethiopia (Desalegn *et al.*, 2015). The country's environmental condition and vegetation are highly conducive (favorable) for ticks and tick-borne disease perpetuation. The life of ticks depends on the host animal which results in retardation to animal growth, loss of milk and meat

production. Generally ticks could be affecting the market price and decreasing the annual income of humans (Jelalu *et al.*, 2016). Tick distribution and their population in the country vary according to their adaptability to ecology, eco-climate, microhabitats, ambient temperature, rainfall and relative humidity which is critical factors affecting life cycle of ticks. The relative humidity on the other hand remains an important factor for survival of ticks by regulating the water balance and prevents dehydrations (Tadesse *et al.*, 2012). In Ethiopia, ticks and tick-borne diseases in cattle population cause serious economic loss to smallholder farmers, the tanning industry and the country as a whole through the mortality of infected animals, decreased production, down grading and rejection of hide (Getachew *et al.*, 2014). Various studies have been conducted in different localities of Ethiopia. The prevalence of the previous studies have shown a range of 23-85%. However, limited information has been made in the present study areas. Therefore, the objectives of the present study were:

- To estimate the prevalence of hard tick infestation on cattle in the selected districts
- To identify the associated risk factors of tick infestation in the study districts
- To determine the burden of tick infestation in local and cross breeds of cattle
- To identify the species of dominant ticks found in the study areas

## 2. LITERATURE REVIEW

### 2. 1 Taxonomy of Ticks

Ticks are classified under the class *Arachnida*, Order *Acarina*, suborder *Ixodida*, families *Ixodidae* and *Argasidae* which are distributed worldwide. Ticks are members of the same phylum (Arthropoda) of the animal kingdom as insects, but are in different classes (class *Insecta* includes Flies, Fleas and Lice, but class *Arachnida* includes Mites and Ticks). The subphylum *Chelicerata* includes the class *Arachnida*, which again contains several subclasses (Walker *et al.*, 2003). The subclass *Acari* (*syn. Acaria, Acarina, Acarida*) includes ticks. There are two well established families of ticks, the *Ixodidae* (hard ticks) and *Argasidae* (has hard scutum, male and female easy distinguished, collects on the host, each parasitic stages feed only one times, sexual dimorphism is marked, can mate on the host except *Ixodes* spp, mouthparts are visible and has one nymphal stage) or soft ticks (no scutum, male and female not easy distinguished, mostly not collected on the host,) nymphs and adults feed many times, sexual dimorphism not marked, has several nymphal stage active throughout the year and has long life time. Family *Ixodidae* (hard ticks) contains (684) species under many genera. These include *Amblyomma* (102 species), *Boophilus* (5 species) *Dermacentor* (30 species) *Haemaphysalis* (155 species) *Hyalomma* (30 species), *Ixodes* (254 species) and *Rhipicephalus* (70 species) (Horak, 2009).

The *Ixodidae* (hard ticks) and *Argasidae* (soft ticks) both have sharing certain basic properties; they differed in many structures, behavioral, physiological, feeding and reproduction pattern. Ticks that are considered to be most important to domestic animals' health in Africa comprise about seven genera and forty species. Among these tick genera, the main ticks found in Ethiopia are *Amblyomma* (40%), *Boophilus* (21%), *Haemaphysalis* (0.5%), *Hyalomma* (1.5%), and *Rhipicephalus* (37%), *A. variegatum* and *B. decoloratus* are most important and widely distributed in Ethiopia (Walker, 2004).

## 2.2 Ecology of Ticks

Tick habitat is composed of the variety of living and non-living things in the space in which it lives. Ticks are adapted to two contrasting components of their habitat, the **physical environment and their host**. When ticks are moulting and then questing in the physical habitat they are in danger of drying out and starving. The larvae are most susceptible because of they have a high surface area relative to their small volume. They are also exposed to predators such as rodents, birds, reptiles and ants and to pathogens such as fungi. These adverse factors limit the type of habitats that a species will be found in and knowledge of the typical physical habitat of a species is an aid in identification. The most important component of the physical habitat of a tick is the climate that is defined by temperature and humidity (Latif & Walker, 2004)

## 2.3 Habitat of Ticks

A tick's habitat is composed of the variety of living and nonliving things in the space in which it lives that are good or bad for its survival. Ticks are adapted to two extremely contrasting components of their habitat, the physical environment and their host. When ticks are moulting and then questing in the physical environment, which are in danger of drying out starving and freezing. They are also exposed to predators such as ants and to pathogens such as fungi. These adverse factors limit the type of habitats that a species will be found in and knowledge of the typical physical habitat of a species is an aid to identification (Walker *et al.*, 2003).

### 2.2.1. Ixodidae (hard ticks)

There are three active stages in the life cycle of a hard tick: larvae, nymphs and adult ticks. Each instar takes a blood meal only once and long periods are spent on vegetation between blood meals. Most ticks require three different hosts to complete one full cycle. These three-host ticks detach on completion of feeding, drop from the host, molt and wait for another host. However, in some tick species, the engorged larvae remain on the host, where they molt rapidly to become nymphs, continue to feed and then drop as engorged nymphs (Walker, 2004). These two-host ticks include *Rhipicephalus evertsi.evertsi* and some *Hyalomma* species. In one-host ticks, the nymphs also remain on the same host and continue to feed as adults. *Boophilus* spp. are typical one-host ticks

.After the female drops from the host, she seeks a sheltered place for oviposition, where she lays a single batch of several thousand eggs and then dies. Males usually remain much longer on the host, where they may mate repeatedly (Walker *et al.*, 2003).

### 2.2.2 Argasidae

The life cycle and feeding pattern of the soft ticks are different from those of the hard ticks. The *Argasidae* are multi-host ticks; there are several nymph stages and the adults also feed repeatedly. Feeding can last from a few minutes to hours, or even days for the larvae of some species. Most *argasid* ticks live in nests or burrows, although there are exceptions. Adults usually mate in the nest or burrow. Mated females take small, repeated blood meals to support the production of small batches of eggs. The occurrence of several nymph instars and frequent adult blood meals contributes to an unusually long life span (several years) and high resistance to starvation. These species are extremely hardy and can survive in hot, dry conditions for long periods without a blood meal. *Argasid* ticks also concentrate their blood meal by eliminating excess water via the coxal apparatus, which is located in the proximal part of the front pair of legs. There are approximately 170 species of soft ticks. Species of medical or veterinary importance belong to the genera *Argas*, *Ornithodoros* and *Otobius* (Walker *et al.*, 2003).

## 2.5 Biology of Ticks

Ticks are among the most significant blood-sucking arthropods and distributed worldwide. They transmit various pathogens that can cause disease and death in cattle. Ticks have several morphologic features and physiologic mechanisms that facilitate host selection, ingestion of vertebrate blood, mating, survival and reproduction (Berihun *et al.*, 2016). Although the natural history of ticks varies considerably among species, these arthropods are well-adapted to survive in tropical, temperate, and even subarctic habitats. Most ticks require three different hosts to complete one full cycle. These three-host ticks detach on completion of feeding, drop from the host, molt and wait for another host. The life cycle of tick involves according to feeding habitat and characteristic number of host individuals (Walker *et al.*, 2003).

In the hard ticks mating takes place on the host, except with *Ixodes* where it may also occur when the ticks are still on the vegetation. Male ticks remain on the host and will attempt to mate with many females where as they are feeding. The lifecycle of ticks (both *Ixodids* and *Argasids*) undergo four stages in their development (Figure 1); eggs, 6-legged larva, 8-legged nymph and



adult (Walker *et al.*, 2003). According to the numbers of hosts, *Ixodids* ticks are classified as one-host ticks, two-host ticks, three-host ticks and *Argasids* classified as multi-host ticks. In one-host ticks, all the parasitic stages (larva, nymph and adult) are on the same hosts; in two-host ticks, larva attach to one host, feed and molt to nymph stage and engorged, after which they detach and molt on the ground to adult; and in three-host ticks, the larva, nymph and adult attach to different hosts and all detach from the host after engorging, and molt on the ground. In multi-host ticks (*Argasids*), a large number of hosts are involved and it is common to have five molts, each completed after engorging and detaching from the hosts (Be Evans *et al.*, 2000).

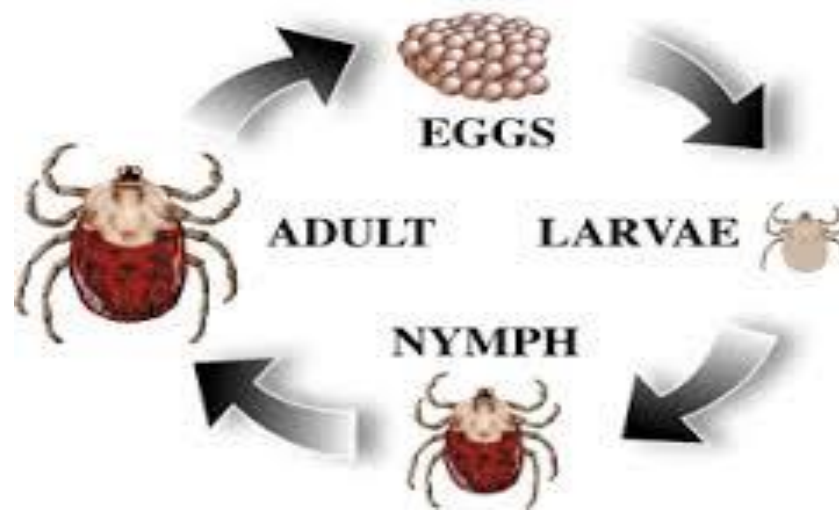


Figure-1: Life cycle of ticks (Walker *et al.*, 2003)

## 2.6 Host Finding

All ticks spend most of their life cycle away from their hosts (without host), hiding either in soil and vegetation or in the nests of their hosts. So they need to be able to find hosts on which to feed. Ticks do this in several ways. Many ticks have the eggs and molting stages in soil or vegetation in the environment in which their hosts graze or hunt. The survival of a population of ticks depends on the presence of hosts suitable for reproduction by the adults. These hosts are known as maintenance hosts. These hosts are more limited in variety than the hosts on which larvae and nymphs of three-host ticks can survive. They are also more limited than those on which adults may attempt to feed but not necessarily survive. (Walker *et al.*, 2003).

### 2.6.1. One-host tick

Cattle tick (*Boophilus*) is a single-host species. The parasitic stage of the tick life cycle (the stage spent on an animal) is spent entirely on a single host. The parasitic stage of the life cycle involves 3 phases; larvae, nymph and adult. Cattle ticks undergo a moult on the host between the larval, nymph and adult phases. During the moult, the tick sheds the previous skin or shell, in order to emerge into the next life stage (i.e. larvae moult into nymphs, and nymphs moult into adults). The parasitic stage begins when a larval tick climbs from the pasture onto an animal. The parasitic stage ends when a fully engorged female detaches from the animal and falls to the pasture (Jelalu *et al.*, 2016).



**Figure 2:** *Boophilus decoloratus* (Jelalu.,*et al.*,2016)

### 2.6.2. Two-host tick

In these species, the larval and nymph stages are spent on the same animal, but the nymph drops off to molt to the adult stage, which then seeks a final host. A few species in the genera *Hyalomma* and *Rhipicephalus* are experienced two-host life cycle (Nateneal *et al.*, 2015).



**Figure 3:** *Rhipicephalus evertsi evertsi* (Jelalu.,et al.,2016))

#### 2.6.3. Three-host tick

Three-host tick life-cycle showing relative sizes of the instars and unfed and engorged ticks, approximately 4 times life size (*Rhipicephalus appendiculatus*). The fed male has not expanded but shows a caudal appendage. *Hypostome* and *palps*, ventral view showing palps spread out to enable only the *hypostome* and chelicerae to penetrate the host. Engorged nymph of *Amblyomma* compared with engorged female of *Boophilus* sub-genus, ventral view. The two species shown may occur at the same time on the same cattle. They can be distinguished by the shape of their mouthparts (Walker *et al.*, 2003).



**Figure 4:** *Amblyomma varigatum* (Jelalu.,et al.,2016)

## **2.7 Epidemiology of Ticks**

### **2.7.1 Host relationship**

Some ticks live in open environments and crawl onto vegetation to wait for their hosts to pass. This is a type of ambush and the behavior of waiting on vegetation is called questing. Thus in general such as *Rhipicephalus*, *Haemaphysalis* and *Ixodes* the larvae, nymphs and adults will quest on vegetation. The tick grabs onto the host using their front legs and crawl over the skin to find a suitable place to attach and feed. Adult tick of genera *Amblyomma* and *Hyalomma* are active hunters, they run across the ground after nearby hosts (Abdela *et al.*, 2016).

## **2.8 Pathogenic Effects of Ticks and Tick-Borne Diseases**

Infested cattle lose condition due to 'tick worry' and loss of blood. Heavy infestations can kill calves and even adult cattle. Animals in poor condition are especially vulnerable (susceptible). Previously unexposed cattle become heavily infested until they build up a degree of resistance. *Bos Indicus* (tropical breeds of cattle) and their crosses, develop a greater degree of resistance than *Bos Taurus* (British and European breeds of cattle). Cattle ticks transmit the organisms that cause tick fever, which is a serious blood parasite disease of cattle. It also causes lower reproductive &

productive efficiency of their hosts. Economic loss through morbidity & mortality of animal hosts and control cost. Hides of infested cattle are damaged by tick bites, reducing their value. In severe cases the hides may be unsalable (Walker 2004).

Especially ticks with long mouthparts cause considerable direct damage to hides of cattle. Secondary infections can cause septic wounds or abscesses, and lesions on the teats of cows may affect milk production. Ticks also have adverse effect on livestock in several ways and parasitize a wide range of vertebrate hosts and transmit a wide variety of pathogenic agents than any other group of arthropod (Walker *et al.*, 2003).

## **2.9 Consequences of Infestation**

Ticks present three main dangers to their hosts: the physical damage from the bite itself, other systemic effects of the tick's saliva and transmission of infectious diseases. When ticks attach themselves to the host in the first stage of feeding, they cut the skin with their mouthparts and cause damage to tissues and capillaries. Host reactions, such as mast cell degranulation leading to histamine release and inflammatory cell infiltration, further contribute to tissue damage. This tissue damage tends to be quite painful and may result in secondary bacterial infections. (Shearer, 2010).

### **2.9.1 Direct effect of ticks**

Ticks are responsible for direct damage to livestock through their feeding habits. The damage is manifested as hide damage, damage to udders, teats and scrotum, myiasis due to infestation of damaged sites by maggots and secondary microbial infection. Ticks bite can directly debilitating to domestic animals, causing mechanical damage, irritation, inflammation and hypersensitivity (Abdela *et al.*, 2016).

Feeding by large numbers of ticks causes reduction in live weight and anemia among domestic animals, while tick bites also reduce the quality of hides. Apart from irritation or anemia in case of heavy infestations, tick can cause severe dermatitis. These parasites generate direct effects in cattle in terms of milk production and reduce weight gain (Tiki and Addis, 2011).

### 2.9.2 Physical effect of ticks

Ticks are attached to the body for a blood meal and may cause irritation and serious physical damages to livestock. Included tick worry, irritation, unrest and weight loss due to massive infestation of ticks. The direct injury to hides due to tick bites, loss of blood due to the feeding of ticks. When cattle are heavily infested, ticks can be found anywhere on the body of animals (Walker, 2004).

### 2.9.3 Vector of pathogens.

The main diseases transmitted by ticks to livestock are *anaplasmosis* (ruminants), *babesiosis* (ruminants, horses, and dogs), *theileriosis* (ruminants, horses) and *cowdriosis* (ruminants). *Ehrlichiosis* in ruminants and dogs is also important in certain tropical and subtropical regions. Endemic stability can often be achieved, especially in indigenous livestock and even East Coast fever may cause no more than slightly increased calf mortality in local zebu in fully endemic areas. *A. variegatum* (vector of *Cowdria ruminantium* and *Theileria mutans*) and *Boophilus* species (vector of *Anaplasma marginale* and *Babesia bigemina*) are the most widespread ticks in Ethiopia. *R. evertsi evertsi* is also known to occur across different ecological zones of the country serving as a vector for *Babesia bigemina* in cattle, (Ferede, *et al.*, 2010). Estimates of economic losses due to ticks and tick-borne diseases are often little more than educated guesses. Any form of control in local resistant livestock is not always cost-effective, whereas intensive and expensive control measures are often required for valuable exotic breeds (Latif and Walker, 2004).

#### *Babesiosis*

*Babesiosis* is an emerging, tick-transmitted, zoonotic disease caused by hematotropic parasites of the genus *Babesia*. *Babesia* parasites (and those of the closely related genus *Theileria*) are some of the most ubiquitous and widespread blood parasites in the world, second only to the *trypanosomes*, and consequently have considerable worldwide economic, medical, and veterinary impact. The parasites are intraerythrocytic and are commonly called *piroplasms* due to the pear-shaped forms found within infected red blood cells. The major species of *Babesia* which causes *bovine babesiosis* are *B. bovis*, *B. bigemina*, *B. divergens* and *B. major*. *Babesia* parasites can be transmitted transovarially between tick generations in the case of *Ixodes*, surviving up to 4 years

without a vertebrate host. *Babesia* may also be transmitted by fomites and mechanical vectors contaminated by infected Mary *et al.*, (1996)

### *Anaplasmosis*

*Anaplasmosis*, formerly known as *gall sickness*, traditionally refers to a disease of ruminants caused by intraerythrocytic organisms of the order of genus *Anaplasma*. *Anaplasmosis* is a vector borne infectious blood disease in cattle caused by the *rickettsial* parasites. It is not contagious, but numerous species of tick vectors (*Boophilus*, *Dermacenter*, *Rhipicephalus*, *Ixodes* and *Hyalomma*) can transmit *Anaplasma* species. It causes outbreaks in a herd, which can lead to the death of adult cattle. Other economic losses include abortions, decreased weight gain, bull infertility and treatment costs. Although many outbreaks of *anaplasmosis* occur in the spring and summer, they can occur at any time of the year (Latif and Walker, 2004).

### *Theileriosis*

*Theileriosis* results from infection with protozoa in the genus *Theileria* of the suborder *Piroplasmorina*. *Theileria* species are obligate intracellular parasites. *Theileria* is considered to be spread by bush ticks and/or introduction of infected animals from an endemic area. It may also be spread via standard husbandry practices that include blood transfer (such as using needles on multiple animals) and across the placenta. Bush ticks only transfer *Theileria* to cattle, not other species (Vivian, 2010).

#### 2.8.4 Tick bite paralysis

It is characterized by an acute ascending flaccid motor paralysis caused by the injection of a toxin by certain ticks while feeding. Examples are paralysis caused by the feeding of *Dermacentor andersoni*, sweating sickness caused by *Hyalomma truncatum*, tick toxicosis caused by *Rhipicephalus species* and other tick paralysis caused by *Ixodes holocyclus*. Effective diagnosis of tick borne *hemoparasitic* diseases of ruminants is helpful to implement appropriate prevention and control strategies. Tick control, chemoprophylaxis and immuno-prophylaxis are the basic methods to control tick borne hemoparasitic diseases of ruminants. (Latif and Walker, 2004). Housing in tick proof buildings, Separate housing of cattle from others, Quarantine, Pasture spelling and rotational grazing, Manual removal of ticks, Clearance of vegetation, Use of acaricides, Use of

biological control methods( e.g. birds, rodents, shrews, ants and spiders play some role in tick control measures),Breeding cattle for tick resistance, Ethno veterinary practices against ticks (e.g. Several plants and herbs have been shown to possess anti-tick insecticidal, growth inhibiting, anti-molting and repellent activity and Tick vaccine (Vivian, 2010).

## **2.10 Control Methods**

### **2.9.1 Biological control methods of ticks**

Ticks have numerous natural enemies, but only a few species have been evaluated as tick bio-control agents. Some laboratory results suggest that several bacteria are pathogenic to ticks, but their mode of action and their potential value as bio-control agents remain to be determined (Jongejan, 1994).

Natural enemies of ticks include insectivorous birds, parasitoid wasps, nematodes, *Bacillus thuringiensis* bacteria, and deuteromycete fungi (largely *Metarhizium anisopliae* and *Beauveria bassiana*). The potential of each of these taxa as bio-control agents will be discussed in turn. Mammals and birds typically consume ticks during self-grooming. ( Jongejan and Uilenberg, 1994)

### **2.10.2 Tick vaccine**

Tick infestations affect animal health and production worldwide, both for the impact on animal weight gain and milk production and for the pathogens transmitted by these ecto-parasites. *Acaricides* are a major component of integrated tick control strategies, but their application had limited efficacy in reducing tick infestations and often accompanied by serious drawbacks, it includes the selection of acaricide-resistant ticks, environmental contamination and contamination of milk and meat products with drug residues. (Jongejan, F. and G. Uilenberg, G.1994) .All of these issues reinforce the need for alternative approaches to control tick infestations and pathogen transmission that is the use of vaccines (a vaccine which is prepared from infected ticks) with tick antigens (Merino et al., 2013).



### 2.10.3 Breeding cattle for tick resistance

For many years, acquired resistance to ixodid ticks has been recognised as a possible biological control method. Such resistance, acquired after repeated infestations by ticks, is immunologically mediated. Acquired immunity is expressed by a reduction in the number of ticks which attach to the host, reduced engorgement weights, and reduced egg and larval production resulting in significantly reduced tick populations. (Jongejan, . and Uilenberg, 1994).

Although some of the observed variation in natural tick resistance is related to environmental factors and significant component of variation in natural disease resistance appears to be genetic origin. Several studies have been conducted on genetic determination of tick resistance. Tick resistance has been shown to be heritable reported a heritability estimate of 34% for tick resistance, indicating that genetic improvement through selection should be effective. Resistance of cattle to tick infestation was reported to consist of innate and acquired components (Abdela *et al.*, 2016).

### 2. 10.4 Stock breeding and pasture management.

Cattle breeds indigenous to Africa, typically Bos Indicus or zebu have a good heritable ability to acquire natural resistance to the feeding of ticks. This characteristic can be used in breeding programmed to produce crosses with more productive exotic cattle of the Bos Taurus type which will give good resistance to ticks and good production (Latif and Walker, 2003).

### 2.10.5 Application of chemicals methods

The use of acaricides in the control of ticks has improved the viability of cattle farming in the tick infested areas. Ticks can be killed by dipping or spraying cattle with an appropriate chemical (acaricides). Ticks can develop resistance to acaricides (Blackwell science, 2001).

#### Dipping

In this method, animals are immersed in a dipping tub containing solution of chemicals. Infested cattle should be dipped in the organophosphate acaricide coumaphos (0.3% active ingredient. In

general dipping vats provide a highly effective method of treating animals with acaricides for tick control (Latif and Walker, 2003).

### Spray

The application of fluid acaricides to an animal by means of a spray has many advantages and has been successfully practiced for controlling ticks on most of the animals (Barnett, 1961). Spraying equipment is highly portable, and only small amounts of acaricides need to be mixed for a single application. However, spraying is generally less efficient in controlling ticks than immersion in a dipping vat because of problems associated with applying the acaricides thoroughly on all parts of the animal body (Walker *et al.*, 2003)

### Spot treatment or hand dressing

There are predilection sites for certain tick species on part of the body which are not effectively treated by spray or dips. The inner parts of the ear, under part of the tail, the tail brush and the areas between the teats and the legs in cattle with large udder are especially liable to escape treatment. The application of insecticides with aerosols and in oils, smears, and dusts by hand to limited body areas is time-consuming and laborious, but in certain instances it may be more effective and economical (in terms of cost) of acaricides than treating the entire animal (Blackwell science, 2001)

### The future of tick control in the communal areas

Indigenous Sanga and Zebu cattle which are predominantly reared by communal farmers have a high degree of tick and tick-borne disease resistance and require minimal tick control methods. This tick control method is suitable and cost effective (minimize) for usages, even farmers Kaur *et al.*, (2015)

### Some other control methods and applications

Ticks are commonly controlled by using conventional synthetic acaricides, however it has certain drawbacks like high cost, non biodegradable, toxic to environment, left residuals in animal body and above all development of resistance in ticks. Therefore, the search for herbal alternatives is ongoing process and various researchers are exploring different genera of plants to find extracts with acaricidal properties that can be used in association with or even as an alternative to synthetic

compounds Some other methods of applying acaricides are ear tags, neck bands, tail bands and pour-on, particularly for the pyrethroids with long residual activity (Abdela *et al.*, 2015, Kaur *et al.*, 2015).

### 3. MATERIALS AND METHODS

#### 3.1. Study Area

A cross sectional study was carried out during the period from December 2016 to April 2017 in three purposefully selected districts (Chilga, Gondar Zuria and Wegera) of North Gondar Zone, Amhara Region, Northwest Ethiopia. North Gondar Zone is located at 12°39'29.05 N latitude and 37°5'18.14 E longitude. It is characterized by two distinctive seasons; summer rainfall (June to September) and dry season (December to May). (CSA, 2010).

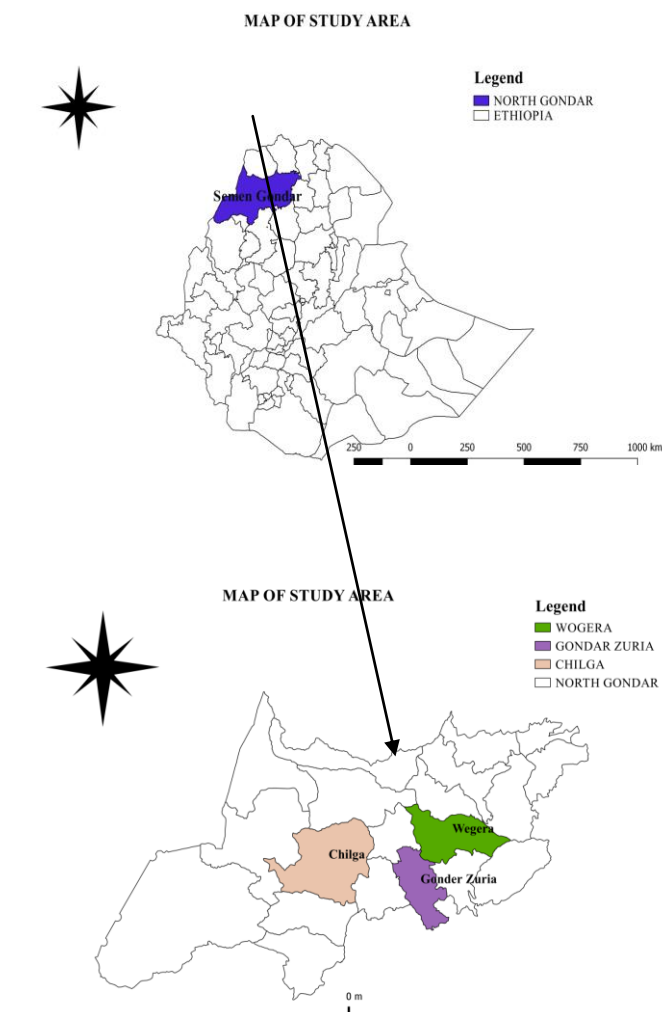


Figure 5: Map of the study districts in Ethiopia

The zone is mostly covered (occupied) by cultivated land, grazing land, forest shrub, bush and woodland, water bodies and the remaining is engaged by settlement population. The livestock population includes 1,936,543 cattle (local and cross breeds), 524,087 sheep, 682,264 goat, other food and non-food producing animals are also available in the zone (Achenafi, 2013). Cattle are particularly important in the agricultural activities or drought power, that means the farmers are dependent on oxen power for crop production (CSA, 2010).

**Chillga:** The majority of the area is made up of a plateau of hilly highlands deeply traversed by steep will be river valleys. The altitude of the area ranges from 1050-2200 m.a.s.l. The agro-ecological zone of the study area includes about 78.3% midland and 21.7% lowland areas (Nibret *et al.*, 2012). The livestock population of the study area is comprised of cattle 181186, sheep 70415, goat 49853, horse 9, donkey 29238, mule 805, poultry 253122 and beehive 11687 (Chillga Agricultural office, 2015).

**Gondar Zuria:** The mean annual rainfall 1800 mm and a mean annual temperature of 20°C. Its altitude is 2300 meters above sea level. The livestock population of the study area is comprised cattle 134,553, sheep 32656, goat 61872, horse 1857, donkey 27541, mule 339, poultry 210147 and beehive 12640). Most populations including Local breed, Cross breed and some them are Exotic breeds occur (Gondar Zuria Agricultural office, 2015).

**Wegera:** The mean annual rainfall 2100 mm and a mean annual temperature of 12 °C. Its altitude is 3810 meters above sea level. The livestock population of the study area is comprised cattle 103708, sheep 59745, goat 65450, horse 4178, donkey 29563, mule 501, poultry 213721 and beehive 7796 (Wegera District Agricultural office, 2015).

### **3.2. Study Population**

The study population was cattle with different age, sex, breed and body condition scores found in the three selected districts. The animals are managed with **extensive management** system and depend on grazing throughout the year for their feed sources with little supplementation of crop residues.

### 3.3. Study Design

A cross-sectional study design was conducted from December 2016 to April 2017 to determine the prevalence of ticks' infestation and to identify associated risk factors and the tick species prevalent in the study districts. A simple random sampling method was employed to select the study individual animals. The study animals were categorized into two groups based on age as young ( $\leq 2$  years) and adult ( $>2$  years) (Okello-Onen, 1999) and based on breed also grouped as cross and local breed, while body condition score was employed after categorizing the animals into poor, medium and good. Accordingly the considered risk factors in the present study were sex, age, body condition score (good, medium and poor), and month and agroecological zones were classified according to (CSA 2013).

### 3.4. Sample Size Determination

The desired sample size was determined by assuming 50% expected prevalence of tick infestation at 95% confidence interval and 5% absolute precision. Therefore, the relevant formula for the desired sample size was based on Thrusfield (2005) as follows:

$$N = Z\alpha^2 / d^2 (P_{exp} (1-P_{exp}))$$

Where:

$Z\alpha$  = the Z (normal distribution) value for a given confidence level (e.g. 1.96 for 95% confidence)

N = required sample size

$P_{exp}$  = expected prevalence

d = desired absolute precision at 5%.

### 3.5. Tick Collection, Identification and Counting

All the selected animals as sampling unit were checked for any tick infestation. The entire body surface of the animal was examined thoroughly for the presence of any tick and all visible adult ticks were collected from **half-body** on alternative sides. Ticks were removed carefully and gently in a horizontal pull to the body surface. The collected ticks were preserved in universal bottles containing 70% ethyl alcohol and labeled with the animal identification and predication site, age, sex, and data of collection. The specimens were transported to the parasitology laboratory of the College of Veterinary Medicine and Animal Sciences, University of Gondar University for

counting and identification. Ticks were counted and subsequently identified to genus and species level by using stereomicroscope, according to standard identification keys given by Latif and Walker (2003) and Walker *et al.* (2003). During examination of the selected animals for tick infestation, the age, sex, body condition score, breed and Kebele of the sampled animals were recorded on a special format designed for this purpose. During the study, distribution of tick and total count of each tick genera were done.

### **3.6. Data Analysis**

All the data were entered on Microsoft Excel spreadsheet and it was analyzed by using statistical package for social science (SPSS) Version 20. **Descriptive statistics** was used to determine the prevalence of tick infestation in cattle. The overall prevalence of tick was determined by dividing the number of positive animals by total sample size and was expressed as percentage. **Chi-square ( $\chi^2$ ) test** was used to point out the possible association of risk factors with the prevalence of tick infestations. **Logistic regression** was used to measure the strength of the associations. Effects were reported as statistically significant in all cases if value is less than 5% at 95% confidence interval (CI).

## 4. RESULTS

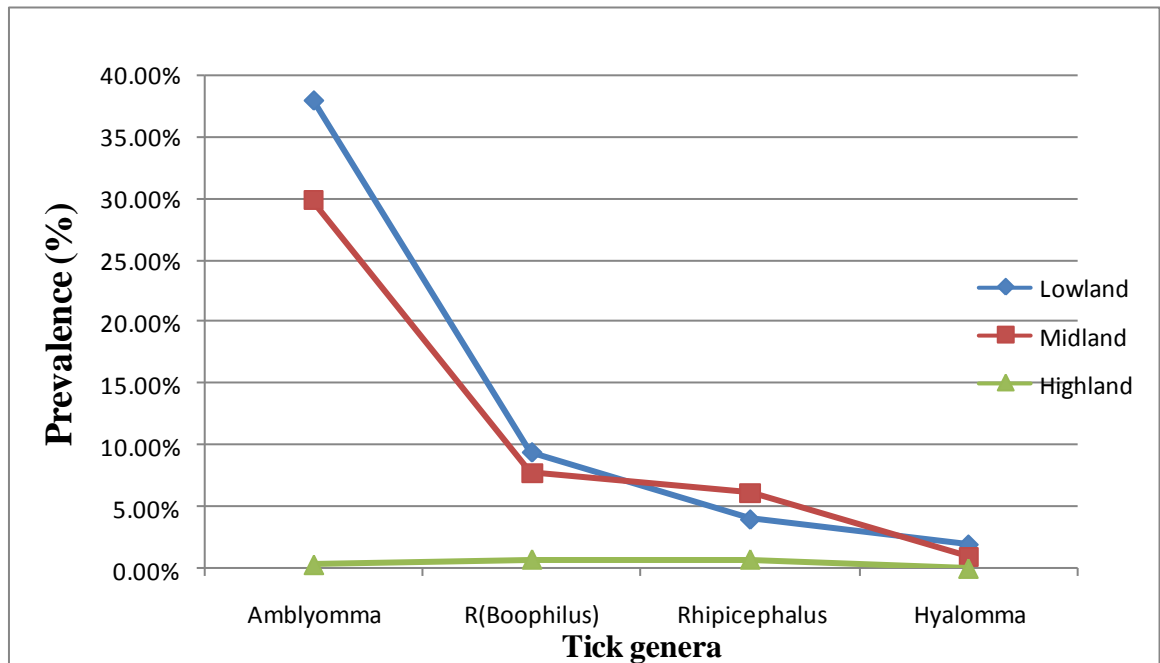
### 4.1. Prevalence, Tick Species and Abundance

Of the total 384 examined cattle, 56% (215/384) were found to be infested by one or more of six species. Examined animals were considered to be positive for a given tick infestation when at least one tick was collected from them. A total of 2161 ticks were collected from 215 examined cattle (Table 1). Out of 2161 ticks collected, 53.4% were collected at lowland and 44.8% were at midland altitude while the least number of ticks were collected at highland (1.8%) (Table 1 and Figure 6). Upon identification, the ticks were classified into four genera and six species. Tick genera recorded in the present study were: *Amblyomma*, *Rhipicephalus* (formerly *Boophilus*), *Rhipicephalus* and *Hyalomma*.

**Table 1:** Univariate logistic regression analysis of tick infestations in cattle

Risk factors		No. examined animals	No. infected animals (%)	Prev. (%)	OR(95% CI)	P-value
Breed	Local	283	135	47.7	1	0.000
	Cross	101	80	79.2	4.18(2.45-7.13)	
Sex	Male	148	80	54.1	1	0.545
	Female	236	135	57.2	1.14(0.75-1.72)	
Age	Young	64	14	21.9	1	0.000
	Adult	320	201	62.8	6.03( 3.2-11.38)	
BCS	Good	54	16	29.6	1	0.032
	Medium	195	90	46.1	2.04(1.06-3.89)	
	Poor	135	109	80.7	10(4.83-20.54)	
Altitude	Highland	128	11	8.6	1	0.000
	Midland	128	98	76.6	34.8(16.6-72.91)	
	Lowland	128	106	82.8	51.3(23.73-110.68)	
Month	February	75	31	41.3	1	0.006
	March	75	33	44	0.41(0.22-0.78)	
	January	75	41	54.7	0.46(0.24-0.78)	
	December	84	53	63.1	0.71(0.37-1.33)	
	April	75	57	76.0	1.85(0.93-3.70)	





**Figure 6:** Distribution of tick genera in different agrological climate

Among the tick species identified, three species (*A. varigatum*, *A. coherence* and *A. lepidum*) were recorded under the genus *Amblyomma*, one species (*R (B). decoloratus*) was from the genus *Rhipicephalus* (formerly *Boophilus*), one species (*R. evertsi evertsi*) from the genus *Rhipicephalus* and one species (*H.marginatum rufipes*) from the genus *Hyalomma* (Table 2).

*Amblyomma varigatum* was the dominant abundantly encountered with high burden followed by *R (B). decoloratus*, *A. cohaerence*, *R. evertsi evertsi*. However, *A. lepidum* and *Hyalomma marginatum rufipes* were the least recorded species on cattle (Table 2). Related with altitude, *A. varigatum* was dominant in lowland and midland, followed by *A. cohaerence*, *R(B). decoloratus* and *R. evertsi evertsi*. However, in the highland altitude, *R (B). decoloratus* and *R. evertsi evertsi* were more frequently encountered tick species (Table 2).

**Table 2:** Abundance of tick species in different agroclimate

Tick species	Altitude			Overall (%)
	Lowland	Midland	Highland	
<i>A. varigatum</i>	576	426	6	1008(46.6)
<i>A. cohaerence</i>	195	190	-	385 (17.8)
<i>A. lepidum</i>	51	29	-	80(3.7)
<i>R(B). decoloratus</i>	204	168	16	388(18)
<i>R. evertsi evertsi</i>	86	134	16	236(10.9)
<i>H. marginatum rufipes</i>	42	22	-	64(3)
<b>Overall (%)</b>	<b>1154(53.4)</b>	<b>969(44.8)</b>	<b>38(1.8)</b>	<b>2161(100)</b>

#### 4.2. Association of Factors With Tick Prevalence

The differences between tick prevalence in cattle per each risk factor categories as well as their associations are summarized in Table 1. During the statistical analyses of all risk factors, the first level of each independent variable was used as a reference category. The result indicated that the prevalence of tick infestation was found higher in cross breed (79.2%) than local breed of cattle (47.7%). The difference was statistically significant (OR= 4.18,  $P < 0.0001$ ). Significantly higher infestation rate of ticks was also observed in adult cattle (62.8%) than young (21.9%) (OR= 6.03,  $P < 0.0001$ ). Similarly, body condition score was significantly ( $P < 0.0001$ ) associated with tick infestation where higher prevalence was observed in poor scored (80.7%) and medium body scored cattle (46.1%) than good body conditioned animals (29.6%).

Further, altitude also significantly ( $P < 0.0001$ ) influenced the prevalence of ticks in cattle; higher prevalence was recorded in lowland areas (at Chilga district) (82.8%) and midland (at Gondar-Zuria) (76.6%) than highland areas (at Wogera) (8.6%). Monthly variation was also noted in tick infestation rate. Higher prevalence was recorded in April, followed by December, January, March and February in descending order (Table 2 and Figure 7). Among considered factors, sex was not significantly ( $P > 0.05$ ) associated with tick infestation rate in the present study area (Table 1).

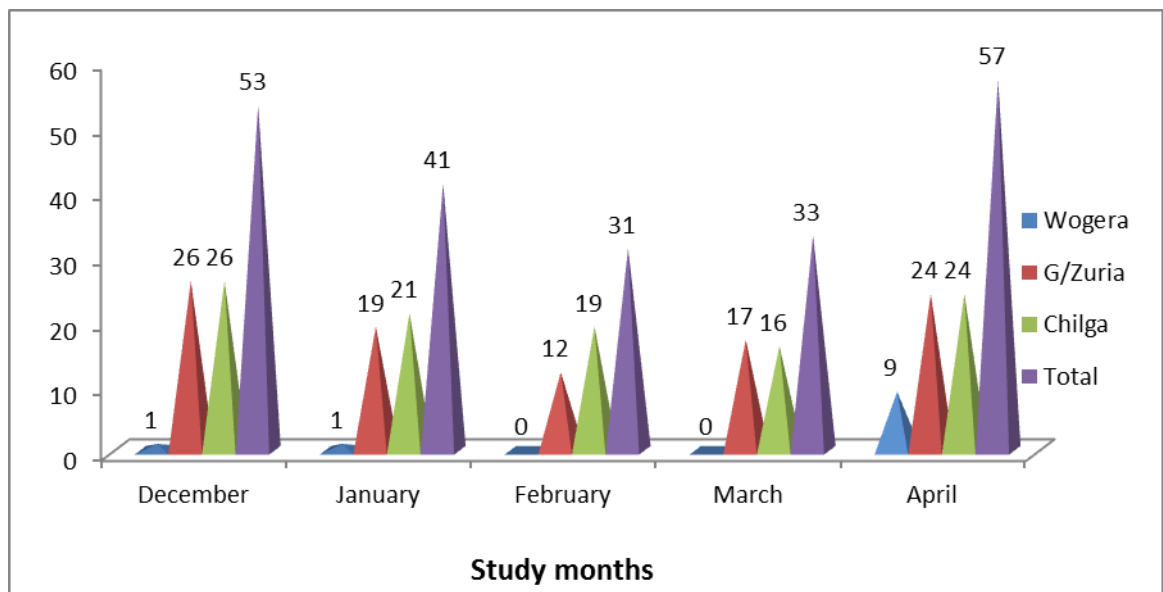


Figure 7: Monthly prevalence of tick infestation

#### 4.3. Distribution of Tick Species and Their Predilection Sites

The distribution of each species of ticks on the host's body is also summarized (Table 3). Ticks were widely distributed in different parts of the host body such as scrotum/udder, groin, dewlap, under-tail, belly, legs/hoof and neck. Groin and scrotul/udder should be the sites of most ticks collected sites followed by dewlap, belly and neck. Under tail and legs/hoof were the least preferred sites by ticks. The most favorable predilection sites for *Amblyomma* species were the udder/scrotum and groin. Moreover, *Boophilus decoloratus* was preferred groin, dewlap, and neck but least number of ticks were also present on the rest of the body. *Rhipicephalus evertsi evertsi* was also preferred more commonly groin and belly.

**Table 3:** Distribution of tick species and their predilection sites

Tick spp	S/u	Groin	Dewlap	Belly	Under tail	Leg/hoof	Neck	Total
<i>A.varigatum</i>	561	302	47	98	-	-	-	1008
<i>A.cohaerence</i>	166	146	-	-	45	28	-	385
<i>R(B).decoloratus</i>	-	173	139	-		-	76	388
<i>R. evertsi evertsi</i>	-	151	33	52	-	-	-	236
<i>A.lepidum</i>	32	25	11	-	12	-	-	80
<i>H.marginatum</i>	21	29	14	-	-	-	-	64
<b>Total</b>	<b>780</b>	<b>826</b>	<b>244</b>	<b>150</b>	<b>57</b>	<b>28</b>	<b>76</b>	<b>2161</b>

S/u =Scrotum or Udder

## 5. DISCUSSION

The distribution and abundance of tick species infesting cattle in Ethiopia vary greatly from one area to another area and also depends on several environmental and climatic factors such as annual rainfall, atmospheric temperature, relative humidity, vegetation cover, altitude and host availability (Pawlos and Deresse, 2013) and Lorusso *et al.*, (2013). In the present study, a total of 2161 adult tick specimens were collected from 215 cattle. Upon morphological identifications, the collected ticks were grouped into four genera and six species. The overall prevalence of tick infestation in this study was found to be 56.0%. This is in agreement with the finding of Getachew *et al.* (2014) in Dembia district, North Gondar Zone, Ethiopia. In the present study *A. varigatum* were found to be the most abundant tick species in 57.1% (in lowland), this is similar to the result indicated in Nibret, *et al.* (2012) in Chilga district, North Gondar Zone. However, lower prevalence was observed in highland (8.60 %). This study agrees with the previous studies on cattle in Assosa and Haramaya by (Bosena and Mohamed 2012) and (Tsegaw *et al.* 2015), respectively. This variation could be due to the difference in the agro-climatic of the study areas. Because the activity of tick is influenced by rainfall, temperature, altitude, a relative humidity and management system include the use of acaricide and other preventive measures (Pawlos and Deresse, 2013).

The identified tick genera in our study include: *Amblyomma*, *Boophilus* (recently *Rhipicephalus*), *Rhipicephalus* and *Hyalomma*. *Amblyomma* (62.6%) was the most abundant and widely distributed ticks in the study period, which coincides with the report of Nigus and Basaznew, (2016), who described 51.24% prevalence of *Amblyomma* in Jabitehnan district, Northwestern Ethiopia. Similarly, Nibret *et al.* (2012) and, Ammanueal & Abdu, (2014) documented *Amblyomma* as a dominant tick in their study sites with a prevalence of 54.9% in Northwest and Southern Ethiopia. Further, Bimrew *et al.* (2015) also reported 37.5% prevalence in Dangila District, Northwest Ethiopia. The observation of high tick counts in lowland agroecology on cattle in the present study is most probably attributed to the vast and seasonal availability of grazing land and unrestricted cattle movement from place to place in the lowland than in both the mid and high agroecological zones (Rahmeto et al., 2010). The study was disagree with Fanos *et al.* (2012) in and around Mizan Teferi, Southwestern Ethiopia, which reported prevalence 18.1%. This might be due to that different species of ticks have different climatic requirements (Bersissa *et al.*, 2012).

In this study, *Hyalomma* (1.2%) was the least recorded tick genus in both breeds of cattle and the three agroclimates. This is in line with the finding of Tegegn *et al.* (2016), who reported 3% prevalence. However, this finding is in disagreement with report of Bimrew *et al.* (2015), who reported a prevalence of 14.4% in Dangila district. This might be associated with the variation between agroclimate and humidity as this work coincides with dry (i.e. from December to April in the area). High humidity facilitates the growth and survival of ticks at all their different life instars (Walker *et al.*, 2003).

The most abundant tick species observed during the study period were *A.varigatum*, *R(B).decoloratus*, *A.cohaerence*, *R.evertsi evertsi*, *A.lepidum* and *H.marginatum rufipes* by descending order. Among these tick species *A.varigatum* (68.43 %) was the most abundant and widely distributed tick species in the study area. This supports the finding of Nigus and Basaznew, (2016) and Nibret *et al.* (2012), who reported a prevalence of 51% in Jabitehnan district and 51.2% in Chilga district, Northwest Ethiopia. Similarly, Bossena and Abdu, (2012) also reported a prevalence of 43.6% in and around Assosa, Western Ethiopia. However, this result disagrees with the works of Morka *et al.* (2014) and Fanos *et al.* (2012), who demonstrated 25% prevalence in Humbo district, Southern Ethiopia and 9% prevalence in and around Mizan Teferi, Southwestern Ethiopia where *R. decoloratus* is the most abundant from the other tick species.

The second observable tick species in the study area was *Rhipicephalus* (formerly *Boophilus*) *decoloratus* (18%). This is in accordance with the previous study of GurMESSa *et al.* (2015), who reported prevalence of 10.7% in and around Sebeta Town. Tsegaye *et al.* (2014), Mohamed *et al.* (2014) also reported a prevalence of 15.5% in Haramaya and 26.3% in Oromia respectively. However, our report is inconsistent with the previous researchers report (Tsegaw *et al.*, 2015) which showed a prevalence of 47.8% at Haramaya District, Eastern Hararghe, Ethiopia.

The third abundant tick species was *A. cohaerence* (17.8%) identified in the study area. This result strongly disagrees with Getachew *et al.* (2014) and Nibret *et al.* (2012), who reported a prevalence of 5.21% in Dembia district and 1.95% in Chilga district, northwest Ethiopia. Similarly, the finding of the present study disagrees with the finding of GurMESSa *et al.* (2015), who reported 2.4% prevalence in and around Sebeta Town, Ethiopia. This might be associated with climate condition variation which might favour the reproduction and survival of ticks.

*Rhipicephalus evertsi evertsi* was the fourth observed tick species with a prevalence of 11%. This is in agreement with the previous authors who reported 15-16% prevalence (Bossena and Abdu, 2012; Mohamed *et al.*, 2014). However, our report is inconsistent with the report of GurMESSa *et al.* (2015), who described

53.4% prevalence in and around Sebeta. Similarly, Belay and Enyew, (2016) also reported higher prevalence (28.6%) than the present report in Sude district, Ethiopia.

*Hyalloma marginatum rufipes* was the least abundant tick species during the study period. It was comprised 2.95% out of the total tick collected. It is consistent with Gurmessa *et al.* (2015), who confirmed 0.8% prevalence in and around Sebeta Town. Bosen and Abdu, (2012) also reported prevalence of 4.70% in and around Assosa Town. Further, Temesgen *et al.*, (2016) also noted out a prevalence of 4.77% in and around Bishoftu Town, Oromia Region, Ethiopia. But, this report disagrees with Nigus and Basaznew, (2016) and Meaza *et al.* (2014) report that documented 23.5% and 33.1% prevalence in Jabitehnan district, Northwestern Ethiopia. The difference in the prevalence might be due to the geographical difference, breed difference of the study animals and season of the study period (Abunna *et al.*, 2009).

From the study animals in the study period, in poor body condition cattle higher number of tick infestation was observed (80.7%), it was agreed with Belay and Enyew, (2016), they reported prevalence of 100% in Sude district, Arsi Zone, Ethiopia. Because poor body conditioned animals had reduced resistance to tick infestation and they exposed tick infestation during grazing on the field than medium and good body conditioned animals.

Among the study months (from December 2016 to April 2017), higher number of tick infestation was observed in April 76% (57/75), followed by December 63.1 % (53/84) and January 54.7% (41/75). However, in February and March the recorded number of ticks infestation was lower. This is in line with the report of (Nibret *et al.*, 2013). This is due to that the tick infestation is higher in wet season than dry season. The dry season results in lower relative humidity and higher environmental temperature which influences the mortality of ticks due to dessication (Messle *et al.*, 2010).

The proportion of tick infestation was higher in adult (62.81%) cattle as compared to young cattle (21.88%). This was statistically significant ( $P < 0.05$ ) and the higher proportion may be due to outdoor management and long distance movement of adult cattle in searching for feed and water as compared to younger cattle. The present study disagrees with Mohammed *et al.* (2012), who stated in the natural habitat of Sanandaj suburb, Iran, who reported that frequency of tick infestation was greatest in young than adults.

In our study, the most favorable predilection site for *A. variegatum* was scrotum /udder (55.65%), followed by groin (26.96%) and belly (9.72%). Similarly, *A. coherense* was recorded most

frequently from scortum/udder (42.12%), followed by groin (37.92%), under tail (11.69%) and legs/hoof (7.27%). While *R(B).decoloratus* was recoded from groin (44.59%), dewlap (35.82%) and neck (20.36%). Further, *R.evertsi evertsi* was from groin (63.98%), dewlap (13.98%) and belly (22.03%).



## **6. CONCLUSION AND RECOMMENDATIONS**

Tick causes severe direct and indirect impact to the cattle productivity and hide value and thereby reduce the foreign exchange of the country. It also transmits tick borne disease which causes severe loss to the productivity of the animals. This study demonstrated that tick burden in cattle was higher in the present study period with the overall prevalence of 56.0%. Four genera and six species of ticks infesting cattle were identified. This suggests that tick infestation is prevalent and important in cattle population, mainly in the lowland and midland. However, it was noted that tick infestation is less common in highland areas (Wegera district). The prevalence of tick infestation was associated with breed, age, body condition score, month and altitude/agroclimate. Therefore, ticks play major roles in reducing productivity and cause health problems of cattle in lowland (Chilga) and midland (Gondar Zuria). Therefore, based on the above conclusion, the following recommendations are forwarded:

- Further detailed studies on the role of different tick species in causing disease in cattle and their economic consequence to the livelihoods call for serious attention.
- Effective tick control program should be formulated and implemented based on the distribution pattern of ticks and factors responsible for their distribution.
- Identifying tick resistance cattle breed is essential to minimize tick infestation problems in the future.

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## 8. ANNEXES

Annex 1. Age Classification Format  $\leq 2$  young and  $>2$  Adult (Belayneh and Bogale, 2016).

**Annex 2:-**Body condition score classification

BCS	Detailed	Score
Poor	<b>Tail head</b> – deep cavity with no fatty tissue under skin and coat condition often rough. <b>Loin</b> – spine prominent and horizontal processes sharp	1
Medium	<b>Tail head</b> – shallow cavity but pin bones prominent; some fat under skin <b>Loin</b> – horizontal processes can be identified individually with ends rounded.	3
Good	<b>Tail head</b> – fat cover over whole area and skin smooth but pelvis can be felt. <b>Loin</b> – end of horizontal process can only be felt with pressure, only slight depression in loin.	5

Source (Klopčič et al., 2011)

**Annex 3:** Procedures of tick identification

1. Before selecting the sampling unit checked whether the cattle infested by  $\geq 1$  tick(s)
2. The infested cattle were properly restrain
3. Removed the ticks carefully and gently by forceps or manually
4. Put the samples within separated prepared sample bottle (scrotum/udder, groin, dewlap, under-tail, legs/hoof and neck for individual cattle)
5. Preserve the sample bottles with contain 70 % ethanol
6. Labeled with respect to sex, age, breed, body condition scores, districts (localities), site of collection and date of sample collection.
7. Put the samples on the cleaned petridish and adjust by tamp forceps
8. Counted and identified the genera and species level by using stereomicroscope

**DECLARATION**

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any University and that all sources of material used for the thesis have been duly acknowledged.

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This thesis has been submitted for examination with our approval as University Advisors.

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